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14-Color Flow Cytometry to Determine the Contribution of Mitochondrial Mass to Differences in Glycolytic Capacity in Human Immune Cell Subsets

Dequina A. Nicholas, Anna C. Belkina, Elizabeth A. Proctor, Forum Raval, Caroline M. Apovian, Douglas A. Lauffenburger, and Barbara S. Nikolajczyk

Mitochondrial metabolism controls immune cell function, but comprehensive tools to assess human primary immune cell metabolic functions in disease are lacking. We have previously demonstrated that $CD19^+$ B cells are more glycolytic than $CD4^+$ T cells, and that both PBMCs and $CD4^+$ T cells from subjects with type 2 diabetes (T2D) are more glycolytic than their counterpart from BMI-matched non-diabetic controls (ND). However, the contribution of mitochondrial mass to these metabolic phenotypes is untested. Generally, effector $CD4^+$ T cells are glycolytic, whereas regulatory T cells and memory $CD8^+$ T cells rely more on fatty acid oxidation. To assess the contribution of immune cell skewing and mitochondrial mass to the enhanced glycolytic capacity of PBMCs from T2D and resting B cells, we designed a 14-color panel based on common lineage markers and chemokine receptor patterns, in lieu of intracellular cytokine staining, including MitoTracker Green to phenotype 63 total samples from ND subjects, subjects with pre-diabetes, and subjects with T2D. Our samples were run in 5 batches on a BD FACSARIA II SORP. Pre-established panel-specific PMT voltages were tracked using 6-peak Ultrarainbow beads. To normalize MitoTracker Green fluorescence intensity that could vary due to batch effects, each batch included a reference donor PBMC sample that was frozen in multiple aliquots from the same blood draw. With this approach, we could quantify the putative percentages of immune cell populations ($CD19^+$ B cells, $CD8^+$ naïve and memory/effector T cells, CD4+ cells including Tregs and populations enriched in Th1, Th2 and Th17) and the relative mitochondrial mass among these subsets. We found that $CD19^+$ B cells in PBMCs from both ND and T2D subjects had significantly less mitochondrial mass than $CD4^+$ cells. This reduced mitochondrial mass may potentially explain B cells greater usage of glycolysis compared to $CD4^+$ T cells. Of all the CD4+ T cell subsets, Th17 cell consistently had the least amount of mitochondrial mass, potentially indicating that these cells are more dependent on glucose than previously appreciated. Our results validate the rigor of our 14-color panel approach to phenotype T cells subsets and quantify relative mitochondrial mass as well as provide a tool to further explore metabolism in human primary cells and disease.